

Claims

1. An oligonucleotide primer pair for amplifying a human α_{1B} -adrenergic receptor gene, wherein each individual primer is non-self hybridizing, contains at least 15 nucleotides, has a melting temperature within the range of 50°C to 85°C; wherein said primer pair is non-cross hybridizing, anneals to two distinct regions, which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of the pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction.

2. An oligonucleotide primer pair of claim 1, wherein said primer pair amplifies a fragment selected from the group consisting of: region A in Figure 1; and region B in Figure 1.

3. An oligonucleotide primer pair of claim 2, wherein each individual primer of said pair comprises a linear sequence essentially identical to the polynucleotide shown in SEQ ID No:1 5'CGGGGGAAGCAAAGTTTCA3' or SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

4. An oligonucleotide primer pair of claim 2, wherein at least one primer of said pair comprises a linear sequence essentially identical to the polynucleotide shown in SEQ ID No:1 5'CGGGGGAAGCAAAGTTTCA3' or SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

5. An oligonucleotide primer pair of claim 2, wherein each individual primer of said pair comprises a linear sequence essentially identical to the

polynucleotide shown in SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' or SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

5 6. An oligonucleotide primer pair of claim 2, wherein at least one primer of said pair comprises a linear sequence essentially identical to the polynucleotide shown in SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' or SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

10 7. An oligonucleotide primer pair of claim 2 having the nucleotide sequences SEQ ID No:1 5'CGGGGGAAGCAAAGTTTCA3' and SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

15 8. An oligonucleotide primer pair of claim 2, wherein at least one primer of said pair has the nucleotide sequence SEQ ID No:1 5'CGGGGGAAGCAAAGTTTCA3' or SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

20 9. An oligonucleotide primer pair of claim 2 having the nucleotide sequences SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' and SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

25 10. An oligonucleotide primer pair of claim 2, wherein at least one primer of said pair has the nucleotide sequence SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' or SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

11. An oligonucleotide primer pair for amplifying a human β_2 -adrenergic receptor gene, wherein each individual primer is non-self hybridizing, contains at least 15 nucleotides, has a melting temperature within the range of 50°C to 85°C; wherein said primer pair is non-cross hybridizing, anneals to two distinct regions, which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of the pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction.

12. An oligonucleotide primer pair of claim 11, wherein said primer pair amplifies a fragment selected from the group consisting of: region A in Figure 2; and region B in Figure 2.

13. An oligonucleotide primer pair of claim 12, wherein each individual primer of said pair comprises a linear sequence essentially identical to the polynucleotide shown in SEQ ID No:5 5'GAATGAGGCTTCCAGGCGTC3' or SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

14. An oligonucleotide primer pair of claim 12, wherein at least one primer of said pair comprises a linear sequence essentially identical to the polynucleotide shown in SEQ ID No:5 5'GAATGAGGCTTCCAGGCGTC3' or SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

15. An oligonucleotide primer pair of claim 12, wherein each individual primer of said pair comprises a linear sequence essentially identical to the

polynucleotide shown in SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' or SEQ ID No:8 5'TCCTCTAGGACTAAAGCTC3'.

5 16. An oligonucleotide primer pair of claim 12, wherein at least one primer of said pair comprises a linear sequence essentially identical to the polynucleotide shown in SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' or SEQ ID No:8 5'TCCTCTAGGACTAAAGCTC3'.

10 17. An oligonucleotide primer pair of claim 12 having the nucleotide sequences SEQ ID No:5 5'GAATGAGGCTTCCAGGCGTC3' and SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

15 18. An oligonucleotide primer pair of claim 12, wherein at least one primer of said pair has the nucleotide sequence SEQ ID No:5 5'GAATGAGGCTTCCAGGCGTC3' or SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

20 19. An oligonucleotide primer pair of claim 12 having the nucleotide sequences SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' and SEQ ID No:8 5'TCCTCTAGGACTAAAGCTC3'.

25 20. An oligonucleotide primer pair of claim 12, wherein at least one primer of said pair has the nucleotide sequence SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' or SEQ ID No:8 5'TCCTCTAGGACTAAAGCTC3'.

21. A method of amplifying a segment of a human I_{1B}-adrenergic receptor gene of a subject comprising the steps of:

a) providing a biological sample of the subject containing nucleic acid molecules;

5 b) amplifying a segment of the gene by employing an oligonucleotide primer pair of claim 1, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of segments of the gene.

10 22. A method for identifying a genetic variation in a human I_{1B}-adrenergic receptor gene of a subject comprising the steps of:

a) providing a biological sample of the subject containing nucleic acid molecules;

15 b) amplifying a segment of the I_{1B}-adrenergic receptor gene by employing an oligonucleotide primer pair of claim 1, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of amplified segments of the gene; and

c) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step.

20 23. A method for identifying a genetic variation in a human I_{1B}-adrenergic receptor gene of a subject according to claim 22, wherein the sequence analytical step is selected from the group of nucleotide sequencing, single-strand conformation polymorphism assay, allele-specific oligonucleotide hybridization, Southern blot
25 analysis, and restriction endonuclease digestion.

24. A method for diagnosing a disease associated with a genetic alteration of a human α_{1B} -adrenergic receptor gene of a subject comprising the step of:

a) providing a biological sample of the subject containing nucleic acid molecules;

b) amplifying a segment of the α_{1B} -adrenergic receptor gene by employing an oligonucleotide primer pair of claim 1, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of amplified segments of the gene;

c) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step; and

d) determining a correlation of the detected variation between the subject and a control.

25. A method for diagnosing a disease associated with a genetic alteration of a human α_{1B} -adrenergic receptor of a subject of claim 24, wherein the sequence analytical step is selected from the group of nucleotide sequencing, single-strand conformation polymorphism assay, allele-specific oligonucleotide hybridization, Southern blot analysis, and restriction endonuclease digestion.

26. A method of amplifying a segment of a human β_2 -adrenergic receptor gene of a subject comprising the steps of:

a) providing a biological sample of the subject containing nucleic acid molecules;

b) amplifying a segment of the gene by employing an oligonucleotide primer pair of claim 11, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of segments of the gene.

27. A method for identifying a genetic variation in a human β_2 -adrenergic receptor gene of a subject comprising the steps of:

- 5 a) providing a biological sample of the subject containing nucleic acid molecules;
- b) amplifying a segment of the gene by employing an oligonucleotide primer pair of claim 11, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of segments of the gene; and
- 10 c) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step.

28. A method for identifying a genetic variation in a human β_2 -adrenergic receptor gene of a subject according to claim 27, wherein the sequence analytical step

15 is selected from the group of nucleotide sequencing, single-strand conformation polymorphism assay, allele-specific oligonucleotide hybridization, Southern blot analysis, and restriction endonuclease digestion.

29. A method for diagnosing a disease associated with a genetic alteration of a human β_2 -adrenergic receptor gene of a subject comprising the step of:

20

- a) providing a biological sample of the subject containing nucleic acid molecules;
- b) amplifying a segment of the gene encoding said receptor by employing an oligonucleotide primer pair of claim 11, a primer-dependent DNA
- 25 polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of amplified segments of the gene;

- c) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step; and
- d) determining a correlation of the detected variation between the subject and a control.

5

30. A method for diagnosing a disease associated with a genetic alteration of a human β_2 -adrenergic receptor gene of a subject of claim 29, wherein the sequence analytical step is selected from the group of nucleotide sequencing, single-strand conformation polymorphism assay, allele-specific oligonucleotide hybridization, Southern blot analysis, and restriction endonuclease digestion.

10

31. A method for diagnosing a disease associated with a genetic alteration of a human α_{1B} -adrenergic receptor gene of a subject of claim 24, wherein said disease is a cardiovascular disorder.

15

32. A method for diagnosing a disease associated with a genetic alteration of a human α_{1B} -adrenergic receptor gene of a subject of claim 24, wherein said disease is hypertension.

20

33. A method for diagnosing a disease associated with a genetic alteration of a human α_{1B} -adrenergic receptor gene of a subject of claim 24, wherein said disease is a prostatic disorder.

25

34. A method for diagnosing a disease associated with a genetic alteration of a human β_2 -adrenergic receptor gene of a subject of claim 29, wherein said disease is a cardiovascular disorder.

35. A method for diagnosing a disease associated with a genetic alteration of a human β_2 -adrenergic receptor gene of a subject of claim 29, wherein said disease is hypertension.

5 36. A method for diagnosing a disease associated with a genetic alteration of a human β_2 -adrenergic receptor gene of a subject of claim 29, wherein said disease is asthma.

10

002227 44220460